The University of Chicago

THE RABBIT'S LUNG AFTER PHRENICOTOMY AND PNEUMOTHORAX

A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANATOMY

1934

By

CLAYTON G. LOOSLI

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THE RABBIT'S LUNG AFTER PHRENICOTOMY AND AND PNEUMOTHORAX [1]

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FIVE PLATES

INTRODUCTION

If the respiratory portion of the rabbit's lung is lined by a greatly thinned epithelial membrane, as described by various investigators, then this epithelium should become more obvious when the lung is contracted. To accomplish this I reduced the expanded left lungs of healthy, adult rabbits with phrenicotomies and prolonged closed pneumothoraces to a completely collapsed state. After the collapsed lungs were fixed, sectioned and stained by various histologic and cytologic methods, a study was made particularly of the respiratory portions.

According to different authors, the respiratory portion of the mammalian lung may be lined by:

1. A continuous layer of flattened nucleated cells of endodermal origin.
2. A continuous layer of flattened epithelium composed of nucleated cells and non-nucleated placques of endodermal origin.
3. Nucleated cells of mesenchymal origin and non-nucleated placques of epithelial origin.
4. A discontinuous membrane composed of nucleated cells of endodermal origin.
5. A discontinuous membrane composed of cells of mesenchymal origin.

[1] This investigation was aided by a grant of the Rockefeller Foundation to the Biological Sciences Division of The University of Chicago.

6. A discontinuous membrane of septal cells leaving the embryonic origin of these cells an open question.

It is not necessary to give in detail the various views and the authors who hold them on the nature of the respiratory lining of the mammalian lungs, for they have been adequately treated in the works of Oppel ('05), Policard ('26), Lang ('25–'26), Seemann ('31), Miller ('32), and Fried ('34).

MATERIALS AND METHODS

Of the forty-five rabbits used in this investigation, thirty-six were subjected to collapse of their left lungs. Two of the rabbits were then killed daily up to 15 days, then at 20, 30, and 40 days after their phrenicotomies and first pneumothoraces. The animals were apparently healthy, adult rabbits. They were placed under ether anaesthesia and their left phenic nerves were sectioned in the neck with aseptic precautions. The rabbits with the exception of those killed 24 and 48 hours after the phrenicotomies were performed were allowed to recover for 1 day before they were subjected to pneumothoraces. These were produced by injecting air aseptically into the left pleural cavities with a 50 cc. syringe and an 18-gauge hypodermic needle.

The animals which were killed 24 and 48 hours after their phrenicotomies received 120 cc. and 180 cc. of air respectively, immediately following the operations, in order to produce a sudden and complete collapse of the left lungs. The remaining animals each received in their left chest cavities daily air injections of approximately 50 cc. until they began to show respiratory distress. This condition usually developed after the fourth air injection. After this time from 20 to 40 cc. of air were injected every 2 or 3 days in order to maintain a positive intrathoracic pressure in the left cavities, thus keeping the left lungs collapsed.

The vascular systems of the collapsed lungs were congested with blood in the following manner, so that the capillaries in the septa would remain open. The animal to be killed was anaesthetized with ether and the whole front of the chest was

quickly cut away to expose the heart and lungs. The pericardial sac was opened and the pulmonary veins were clamped near their entrance into the left atrium. Following this, the right lung was ligated at its hilum, thus shunting all the blood coming from the right heart into the vascular system of the collapsed lung. The force of the heart beat, which continues from 2 to 4 minutes, fills the vascular bed without rupturing the delicate capillary walls.

An edema of the collapsed lungs of some of these animals was produced by injecting intravenously, by way of the femoral vein, 500 cc. of physiological salt solution into each animal a few minutes before it was to be killed in an attempt to separate the respiratory lining from the alveolar walls. The pulmonary vascular systems of these animals were also engorged with blood by ligating the pulmonary veins, as previously described.

For controls, expanded rabbit lungs which had previously been congested with blood in the manner given above, were studied.

The lungs of all the animals were carefully removed in toto and fixed for 18 hours in Zenker-formol solution (nine parts of Zenker stock solution to one part of neutral formalin). The collapsed lungs from three animals were re-expanded by intratracheal injections of fixing fluid. Following fixation, the lungs were washed, passed through iodine-alcohol, dehydrated, and embedded in nitro-cellulose.

Specimens of the lungs were cut in serial sections from 7 to 10 μ in thickness, mounted on slides by the clove oil method, decelloidinized and stained by the following histological methods, alone and in various combinations: 1) Mallory azan for connective tissue, 2) Mallory's phosphotungstic-hematoxylin for smooth muscle, 3) Foot's silver reticulum stain, 4) orcein for elastic fibers, and, 5) hematoxylin-eosin-azure II for cytological detail.

In addition, three animals were killed 8, 10, and 12 days after their respective phrenicotomies. Their collapsed lungs were re-expanded by injecting intratracheally 2 per cent silver

nitrate solution, removed in toto from the animals and placed in 2 per cent silver nitrate solution for 6 hours. They were then placed in 10 per cent formalin for 24 hours, after which they were hardened in alcohol and embedded in nitrocellulose. Both thin (10 μ) and thick (60 to 100 μ) sections of the silvered lungs were made and placed in a photographic developing solution to reduce the silver nitrate, washed and counterstained with Mallory's azan and hematoxylin-eosin-azure II stains.

OBSERVATIONS

Observations of lungs in situ. The gross changes taking place in the left lung during the course of complete collapse involve a diminution in size and an increase in intensity of coloration. The collapsed lung appears as an angular, deep red, firm tissue mass, which resembles in color that of the liver and spleen. The surfaces are moist and glistening, and the vesicular appearance normally present in the expanded lung is absent. The collapsed lung is entirely drawn away from the parietal wall, and is approximately the size of the superior lobe of the normally expanded right lung.

When the collapsed lungs are placed in the fixing fluid, they immediately sink, indicating that little air remains in them. The relative volumes of the right and left lungs after they were fixed and hardened in alcohol, were 55 cc. and 5 cc., respectively.

Changes occurring in the lungs during collapse. The rate and degree of collapse of the lungs following phrenicotomy depends on the amount of air injected into the pleural cavities. The lungs of the animals killed 24 hours after phrenicotomy and not subjected to pneumothorax are only partially collapsed. The lungs of the animals which received 120 cc. of air each intrathoracically, immediately following phrenicotomy, show approximately the same degree of collapse (pl. 1) as do the lungs from animals which received, following phrenicotomy, smaller amounts of air over longer periods of time (pls. 2, 3, and 4). Grossly and microscopically, the same general changes are observed both in the acutely and gradually collapsed lungs.

In grossly dissected preparations the various orders of bronchi in the collapsed lungs are greatly contracted in length. The main bronchi are so shortened that they are approximately half the length of those in the normally expanded lung. The diameters of all the bronchi and bronchioles are decreased and many of the bronchioles which do not possess cartilage in their walls are completely collapsed; and grossly, the terminal bronchioles appear to end in a solid mass of tissue.

Microscopically, the walls of the bronchi and bronchioles in the collapsed lungs are considerably thickened. The epithelium of the main bronchi is thrown into wavy folds, and this condition is increased in the smaller bronchi and bronchioles which have undergone a greater degree of collapse. The connective tissue of the lamina propria is also thickened and thrown into papilla-like projections on which the epithelium rests. The cartilage plates situated in the walls of the larger bronchi are drawn together and overlap one another. In some bronchi the cartilage plates buckle on themselves and are pushed into the surrounding lung tissue. The muscle bundles, which in the expanded lung are widely separated by connective tissue, are closely drawn together so that they appear to form a solid muscle layer in the walls of the secondary bronchi and bronchioles.

The respiratory portion of the collapsed lungs loses grossly its vesicular nature and the cut surfaces have the appearance of a solid organ, resembling in color the liver or spleen. Microscopically, the alveolar ducts, alveolar sacs, and alveoli are represented by narrow clefts which branch freely. The alveolar duct walls, which are interrupted by the mouths of the adjoining alveolar sacs and alveoli in the expanded lung, appear much more continuous in the collapsed lung. In many places the lumina are entirely obliterated but the walls of the ducts are indicated by the wavy bands of connective tissue which surround the mouths of the alveoli opening into them. The alveolar septa become thickened and tortuous. The collapsed alveolar spaces are also indicated by the connective tissue in the septa (pls. 1 to 4).

In the collapsed lungs the vascular systems, which I purposely engorged with blood to keep the capillaries in the alveolar septa open, appear well injected (pls. 1 to 4), although the pulmonary arteries and veins are shortened and decreased in diameter, and their walls are also correspondingly thickened. The terminal arterioles, capillaries and venules, on the other hand, are dilated and filled with blood. In some places in all sections of the collapsed lungs, arterioles can be followed into capillaries (pl. 4) and these in turn, into venules, especially in regions under the pleura. In the alveolar septa of the collapsed and control lungs the most characteristic feature is dilated capillaries filled with blood (pls. 1 to 5).

Because the collapsed lungs were fixed in toto, the deeper portions are sometimes not well fixed, and the epithelium in these regions shows beginning autolytic changes. The epithelium lining the bronchial spaces in the series of collapsed lungs shows no desquamation or hyperplasia except in a section through the lower lobe of the 5-day collapsed lung where a small area of hemorrhage and proliferating cells, approximately 2 mm. in diameter, is seen. It is located subpleurally near the anterior margin of the lower lobe, and was probably caused by puncturing the lung with the hypodermic needle when air was injected to produce the pneumothorax. The normal structure of the collapsed lung in this area is replaced by blood-filled spaces. The walls of the spaces do not have the structure of the collapsed alveolar septa seen in other parts of the lung section, for they do not possess capillaries and are covered by cuboidal or flattened, highly basophilic cells, as seen in hematoxylin-eosin-azure II preparations. In some parts of the hemorrhagic area the basophil cells appear in sheets and cords. Many mitotic figures are seen in these cells, indicating active proliferation.

Serial sections through the involved area show that the blood-filled spaces anastomose with one another and are continuous with a normal collapsed branchiole at the margin of the injured area. The basophilic cells lining the blood spaces

and growing in sheets and cords are conntinuous with the epithelial cells of the adjoining bronchiole. A terminal arteriole, showing a recent organizing thrombus, is also involved in the injured area. At the periphery of the hemorrhage are large phagocytic cells filled with red blood cells and pigment granules. The remaining portion of the section presents the usual appearance of the collapsed lung (pl. 2).

Some of the bronchi and bronchioles in the collapsed lungs contain large, epithelioid cells, singly or in groups which possess vesicular nuclei, prominent nucleoli, and varying amounts of dust pigment in their cytoplasms. These cells are morphologically indistinguishable from the free pigment-containing alveolar phagocytes in the passages of the respiratory portions of the expanded and collapsed lungs (pls. 1, 2 and 5). Some areas in the collapsed lung contain more alveolar phagocytes than do others. No mitotic figures are seen in these cells.

The collapsed lungs in which I attempted to produce an edema by intravenous injection of physiological salt solution just before the animals were killed exhibit the same general microscopic appearance as do the non-edematous collapsed lungs (pl. 3). In some areas in the edematous lung sections, the collapsed alveolar ducts and alveoli are partially distended by the edema fluid. In the Mallory-azan preparations a blue-staining precipitate which is similar in color to the blood plasma in the capillaries is present in the alveolar spaces. The blood capillaries in the alveolar septa are greatly dilated, but no membrane has been lifted off their walls by the edema fluid.

Structure of the terminal bronchiole. Because this study is primarily concerned with the structure of the respiratory portion of the rabbit's lung as shown after collapse by phrenicotomy and pneumothorax, a more detailed description of the respiratory portion, beginning with the terminal bronchiole, will be given. In the rabbit's lung it is sometimes difficult to distinguish between terminal bronchioles and respiratory bronchioles because the latter do not always possess alveoli in their walls.

The terminal bronchiole in the collapsed rabbit's lung is lined by a continuous, single layer of epithelium (pl. 4). The epithelial cells may vary in shape from low cuboidal to the simple columnar types. They may be ciliated or non-ciliated. Ciliated epithelial cells may be present up to the termination of the bronchiole. As a rule, the round, oval or elongated nuclei are located in the basal portion of the cells. Each nucleus possesses one or two small nucleoli, and small chromatin particles are distributed evenly throughout the nucleus. The cytoplasm of the epithelial cells has a fine, granular appearance after Zenker-formol fixation.

The epithelium of the terminal bronchioles rests on a basement membrane of fine reticular and elastic fibers (pl. 4). The elastic fibers are closer to the epithelium than are the reticular fibers and run in a longitudinal direction in the wall. The lamina propria is made up of collagenous, reticular and elastic fibers, and of the various types of cells usually found in loose connective tissue. These are embedded in an amorphous ground substance which can be demonstrated with the Mallory-azan stain. Numerous blood capillaries occupy the meshes of the connective tissue fibers.

The muscle bundles do not form distinct layers but are grouped into small fascicles which run in all directions in the connective tissue wall of the bronchiole. Bundles of collagenous and reticular fibers encircle the muscle groups and small branching reticular fibers run between the individual muscle cells (pl. 4). The collagenous connective tissue which surrounds the muscle bundles is continuous with that in the lamina propria as well as that in the adventitial coat. The elastic fibers are less numerous than the collagenous fibers in the connective tissue surrounding the muscle bundles. No cartilage is present in the walls of the terminal bronchioles. Lymphatic vessels are sometimes seen in the loose connective tissue of the adventitial coat. Lymph nodules are not seen at the distal end of terminal bronchiole, but occasionally one is found at the bifurcation of a bronchiole. A small artery accompanies the terminal bronchiole (pl. 4).

The structure of the respiratory bronchiole. The structure of the respiratory bronchiole, with the exception of the presence of alveoli in its walls, is similar to that of the terminal bronchiole. Around the mouths of the alveoli which open into the respiratory bronchiole, the cuboidal epithelium is abruptly interrupted. It may dip into the mouths of the alveoli, but it does not continue as a flattened, pavement epithelium over the surface of the alveolar outpouchings. The reticular basement membrane on which the epithelium rests also discontinues as such. The reticular and elastic fibers of the lamina propria form thick bundles which encircle the mouths of the adjoining alveoli. Small, branching reticular and elastic fibers continue from the larger fiber bundles around the openings of the alveoli into the alveolar walls. These fibers occupy the central portion of the septa and support the capillaries which bulge into the adjacent alveolar spaces.

The structure of the wall of the alveolar duct. Where the respiratory bronchiole branches to give rise to the alveolar ducts, its cuboidal epithelium and reticular basement membrane terminate. The connective tissue of the lamina propria and small muscle bundles continue on to form the walls of the alveolar ducts (pls. 4 and 5). They surround the mouths of the adjoining alveolar sacs and alveoli, and support a network of anastomosing capillaries which are continuous with those in the septal walls. In tangentially cut sections of the alveolar ducts, the connective tissue knobs are sometimes seen to be completely surrounded by capillaries, which are continuous with those in the adjoining septa. The capillaries, connective tissue and smooth muscle cells are embedded in an amorphous ground substance, as seen in the Mallory-azan preparations. The free surfaces of the capillaries are devoid of a continuous cellular covering, but isolated cells can be seen in some of the inter-capillary spaces.

Structure of the alveolar septa. The alveolar septa in the collapsed rabbit's lung, after the capillaries in the septa are filled with blood, are very wide as compared with those in the

expanded lungs (pls. 1 and 5). Their structure is the same in all parts of the collapsed lung. The capillaries are extremely numerous in each alveolar septum and form a complex network which weaves back and forth in the meshes of the reticular and elastic fiber network, forming loops which bulge into the adjacent alveolar spaces (pls. 1 to 5). Only where the capillaries pass from one side of the septum to the other are they completely surrounded by reticular and elastic fibers. In thin sections of collapsed lungs, the capillaries are seen to lie on rather than in the connective tissue stroma of the septa. Their free surfaces are not covered by a reticular basement membrane or a demonstrable epithelium.

The reticular and elastic fibers occupy the centers of the septa, as shown in sections stained with orcein and Foot's silver reticulum stain (pl. 2). They are continuous with the larger bundles of collagenous and elastic fibers in the walls of the alveolar ducts. The reticular fibers are more numerous than the elastic fibers. Occasional small cells which resemble fibroblasts of the loose connective tissue are present along the course of the reticular and elastic fibers (pls. 2, 4 and 5). They do not resemble or suggest smooth muscle cells. The reticular and elastic fibers with these cells and the capillaries are enclosed in an amorphous ground substance which can be demonstrated in sections stained with the Mallory-azan stain.

In some of the spaces between the capillaries of the alveolar septa in the collapsed lungs, are nucleated cells—the septal cells of Lang (pls. 1 and 5). These are the same cells which are seen in the inter-capillary spaces on the surface of the alveolar ducts. The septal cell has a round or oval nucleus which contains a relatively thick nuclear membrane and a prominent nucleolus. The chromatin particles are larger than those of the nuclei of the epithelial cells lining the respiratory bronchioles. The cytoplasm of the septal cell is granular and contains vacuoles which gave it a 'foamy' appearance (pls. 1 to 5). The distribution of the septal cells in the alveolar walls of the expanded and collapsed lungs is very irregular. They are present in some inter-capillary spaces and absent in others.

These cells are as infrequent in the septa of the subpleural alveoli as they are in the walls of those alveoli in the deeper parts of the collapsed lung. In every situation where they occur the septal cells touch or are partially surrounded by the connective tissue stroma of the septa.

The pleura. The pleura of the collapsed lung is greatly thickened and the mesothelial cells are roughly cuboidal. Beneath the mesothelium is a dense layer of elastic fibers under which is another thicker but looser layer of collagenous reticular and elastic fibers, continuous with those in the interlobular septa and in the alveolar walls (pl. 2). The lymphatic spaces in the pleura are numerous and are often seen to be filled with lymphocytes.

Silver nitrate preparations. In the non-counterstained, silvered lung sections a system of black lines can be seen in the walls of the alveolar septa. In thin sections (15 μ) these lines can be followed from one side of an alveolar septum to the other. They can also be followed along the septal walls into the lumina of the venules. Further details of their relationship to other parts of the septa cannot be observed clearly in uncounterstained, silver nitrate preparations. When silvered lungs are stained with hematoxylin-eosin-azure II or the Mallory-azan stains, the relationship of the silvered lines to the cells and connective tissue fibers can be seen.

The silvered lines mark the limits of the endothelial cells of the capillaries in the alveolar septa and follow the capillaries from one side of a septum to the other. The nucleated cells in some of the inter-capillary spaces of the septa are not delimited by the silver but are isolated cells. Where the capillaries of the alveolar septa pass into the venules, the silver lines, marking the limits of the endothelium of the capillaries, are continuous with those which outline the endothelial cells lining the venules. Non-nucleatd placques covering the capillaries of the alveolar septa are not seen in the silver nitrate preparations.

DISCUSSION

Munk (1862), as far as I have been able to trace the history of this subject, was the first investigator to use the method of experimental collapse as a means of studying the structure of mammalian lungs. He performed pneumothoraces on rabbits, after which he injected their vascular systems with silver gelatin and stained the lungs with carmine. From Munk's findings he concluded that the lung alveoli were lined only by the capillary membranes. Recenty, Dogliotti and Amprino ('32) studied experimental atelectatic lungs of guinea pigs and rabbits. These authors maintain that in no case could the nucleated cells outside the capillary walls be found to continue over the capillary loops in the alveolar septa and therefore concluded that a continuous layer of nucleated epithelium lining the alveolar spaces is not confirmed in their experiments.

Chini ('29) and Bettini and Celotti ('33) collapsed the lungs of guinea pigs, rabbits and dogs, either by phrenicotomies or pneumothoraces, and studied the changes by injecting the collapsed lungs intratracheally with vital dyes. From their experiments they concluded that there was a proliferation of cells in the capillary meshes and of free alveolar phagocytes following collapse. Chini considered that the hyperplastic cells which he observed in collapsed lungs belong to the reticulo-endothelial system. Because the attached cells in the capillary meshes did not take the vital stain as did the alveolar phagocytes, Bettini and Cellotti conclude that they are of epithelial origin. The method of vital staining, however, is not adequate as a means of determining if there is or is not a cellular increase in the lungs following collapse, for since the work of Slavjansky (1869) many investigators have shown, as he did, that vital dyes, and, particulate matter including bacteria, when injected intratracheally stimulate the production of free and attached cells in mammalian lungs.

Lang ('25–'26) studied vitally stained tissue cultures of rabbits' lungs and found that the small nucleated cells arose in the connective tissue of the septa. The cells were seen to hy-

pertrophy and migrate into the alveolar spaces and surrounding culture medium and gave rise to phagocytes which took up large quantities of the dye. He also showed by tissue culture methods that these cells take part in the formation of the tubercle after tubercle bacilli were added to the cultures. From his tissue culture studies, Lang concluded that the nucleated cells in the meshes of the capillaries were of mesenchymal origin and called them 'septum cells.' Lang, however, accepted the presence of the non-nucleated placques covering the capillaries in the septa. Policard ('26), Fried ('27–'34), and others, on the basis of their observations following the intratracheal injection of vital dyes, bacteria and particulate matter in the lungs of guinea pigs and rabbits, also conclude that these cells are of mesenchymal origin. These authors saw the nucleated cells hypertrophy, mobilize, and give rise to alveolar phagocytes which ingested large quantities of the foreign material. Seemann ('31), however, observed the same phenomena in the lungs of mice but concluded that the cells which took up the vital dye and other foreign materials are epithelial cells which assume the special role of phagocytosis.

The results of these investigations on experimentally collapsed lungs do not agree with those of W. S. Miller ('32) who, after many years' study of pathological atelectatic and pneumonic lungs of man, supports, with others, the view that the respiratory portion of the mammalian lung is lined by a continuous layer of flattened nucleated epithelial cells. Miller's figures show a detached membrane lining exudate filled alveolar spaces of pneumonic lungs. He also maintains that in pathologic atelectatic lungs, the flattened nucleated cells lining the alveoli round up and become cuboidal in shape, and that only the absence of smooth muscle in the alveolar walls prevents them from being mistaken for sections of bronchioles.

Special care was taken in this study to prevent an inflammation of the collapsed lungs, because any inflammation in mammalian lungs increases greatly the free cells in the lung alveoli. These cells which appear during the inflammatory process, whether the lung be expanded or collapsed,

may fill completely the alveolar spaces or adhere to the surfaces of the septa so that they appear to form a continuous membrane. I do not believe, however, that the histogenesis of these cells can be determined by studying any one phase or stage of the process of inflammation in the lungs, because inflammatory exudate cells from the blood or connective tissue may assume an epithelium-like arrangement, as Huzella ('28) has shown for circulating blood cells and, as may be observed in tissue culture. Therefore, a cellular membrane, present in pneumonic lung alveoli, as Miller finds, is not proof that a membrane exists in healthy lungs free from inflammation.

The only evidence of an epithelium lining the alveolar spaces in the collapsed lungs of healthy rabbits was in the small hemorrhagic area in sections of a 5-day collapsed lung which has already been described. A study of serial sections through this area show clearly that the proliferating, basophilic cells lining the alveolar-like spaces arise from the epithelium of the normal bronchiole ending in this area.

The silver nitrate method of von Rechlinghausen, first applied to the study of the lung by Eberth and Elenz (1864), and later used extensively by Kölliker (1880), is the only method which later investigators, Oppel ('05), Ogawa ('20), Bratianu and Guerriero ('30), Seemann ('31), and others, have used to demonstrate a membrane composed of nucleated cells and non-nucleated placques lining the alveolar spaces of the mammalian lung. Chiodi ('31) and Bloom ('30) have come to doubt the adequacy of this method as a means of demonstrating an epithelium lining the pulmonary alveoli. Chiodi injected silver nitrate intratracheally into the lungs of guinea pigs and rabbits, after the lungs had previously been vitally stained with pyrrol blue, and found that the black silvered lines did not correspond to the limits of the vitally stained cells in the meshes of the capillaries. Bloom has pointed out that in addition to marking the limits of cells, silver nitrate may also stain reticular fibers, fibrin and red blood corpuscles.

My observations made on expanded and collapsed rabbit lungs, which were re-expanded with silver nitrate and counterstained with hematoxylin-eosin-azure II, agree with those of Chiodi. The black lines mark the limits of the endothelial cells of the capillaries in the alveolar septa. The septal cells are not a part of the system of silver lines but are isolated cells in the meshes of the capillaries.

According to those investigators who maintain that the alveoli of mammalian lungs are lined by a continuous layer of epithelium, the alveolar septa have a complex structure. Five distinct layers of tissue are described in the septal walls. They are: two epithelial membranes lining the adjacent alveolar surfaces, two reticular basement membranes which support the epithelial coverings, and in the center of the septum a single network of blood capillaries.

Such a structure of an alveolar septum, however, is not observed in the expanded, and collapsed lungs of rabbits. In these preparations the reticular and elastic fibers form a network which occupies the center of a septum and serves as a framework in and on which the capillaries lie. The capillaries weave back and forth from one side of a septum to the other and form loops which bulge into adjacent alveolar spaces. The free surfaces of the capillary loops which protrude into the alveoli do not possess a reticular covering or a demonstrable epithelium. The reticular and elastic fibers and capillaries are embedded in an amorphous ground substance which can be demonstrated with the Mallory azan stain. The nucleated cells which appear in some of the intercapillary spaces are isolated cells irregularly distributed in the alveolar spaces. By comparing the alveolar septa in the collapsed lungs with those in the expanded control lungs whose vessels are filled with blood, the same structure is observed.

The structure of the alveolar septa which I have observed in the collapsed and control lungs of rabbits is strikingly similar to that described by Todd and Bowman (1859). Although these authors did not observe the septal cells and ground membrane, they did describe clearly the relationship

of the capillaries to the supporting tissue in the alveolar walls of mammalian lungs.

The structure of the respiratory portion of the collapsed rabbit lungs supports one of the recent views on the embryogenesis of mammalian lungs. Dogliotti ('28), Wenslaw ('30) and Lambertini ('33) maintain that in the later stages of lung development the epithelium lining the primordial respiratory spaces becomes discontinuous and forms in the adult lungs the isolated cells seen in the meshes of the capillaries. Rose ('28) and Alice ('33) believe, as a result of their studies of human fetal lungs, that in the later stages of development the epithelium lining the future alveoli degenerates completely and that histiocytes from the connective tissue of the septa come to occupy the spaces between the capillaries and the alveolar walls.

In both collapsed and expanded adult rabbit lungs, nucleated cells can be seen outside the capillaries in the walls of the alveolar ducts, alveolar sacs and alveoli. Their distribution and functional properties have been studied extensively by numerous investigators, but the origin of these cells is still an open question which can be settled only by complete embryogenic studied. At present there is no morphologic or histologic method which can demonstrate that the nucleated cells in the cepillary meshes in the alveolar septa form a continuous histologic epithelium lining the spaces in the respiratory portion of the collapsed or expanded rabbit's lung. Until such a method is found, the presence of a continuous alveolar epithelium derived embryogenically from the entoderm must be denied.

CONCLUSIONS

1. In both the expanded and collapsed lungs of rabbits the alveolar ducts, alveolar sacs and alveoli are not lined by a continuous histologic epithelium.

2. The nucleated cells in some of the intercapillary spaces in the walls of the alveolar ducts, alveolar sacs and alveoli are isolated cells.

3. Non-nucleated placques are not seen.

4. The reticular and elastic fiber networks in the walls of the alveolar ducts and alveoli serve as a supporting structure for the blood capillaries.

5. The reticular and elastic fibers, connective eissue cells, and the blood capillaries are enclosed in an amorphous ground substance which can be demonstrated with the Mallory-azan stain.

I wish to express my thanks to Dr. William Bloom, under whose direction this work was carried out, for the valuable suggestions and generous encouragement he offered throughout this investigation.

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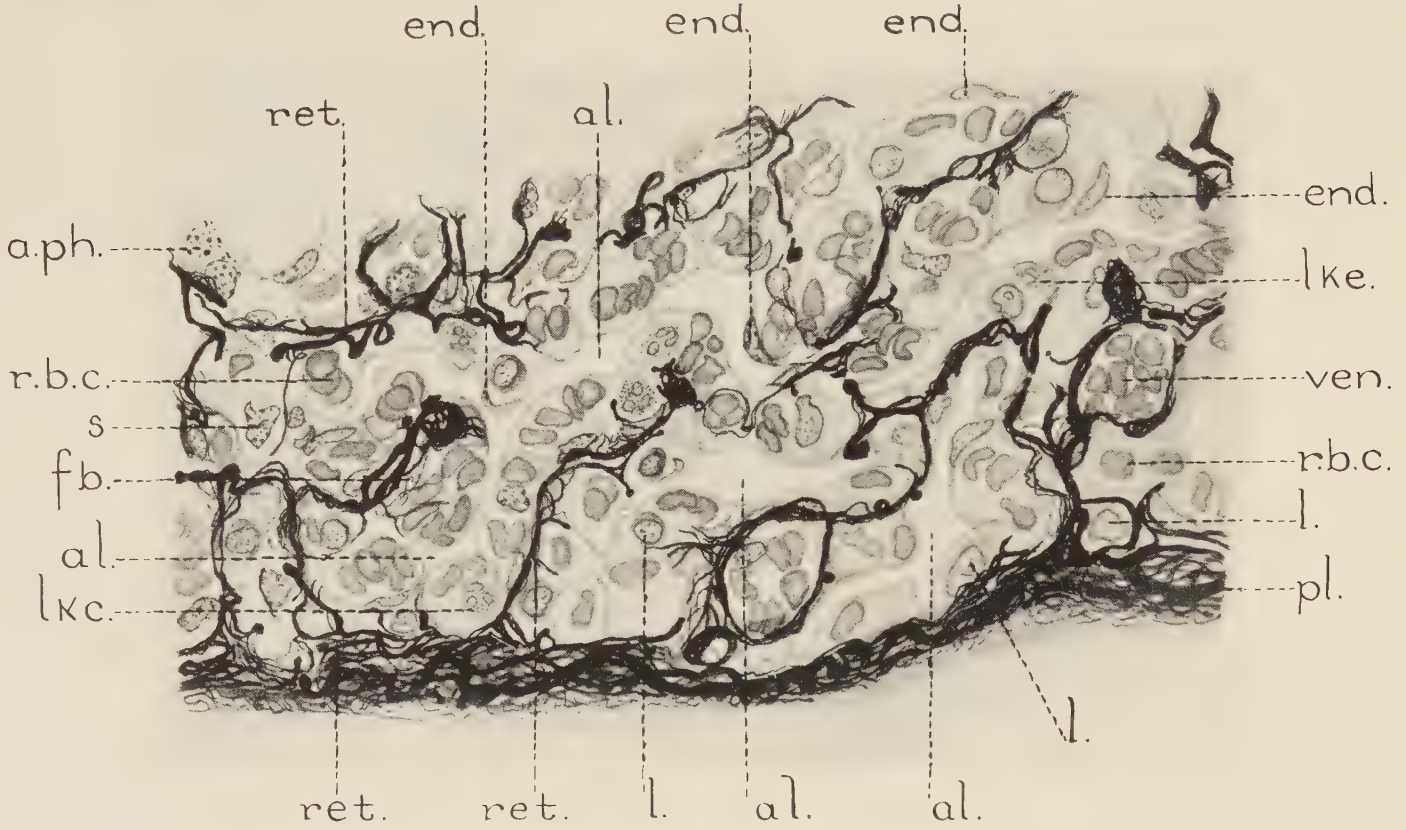
PLATE 1

Section of a collapsed rabbit lung removed 24 hours after phrenicotomy and pneumothorax: al., collapsed alveolus; a.ph., alveolar phagocytes; fb., fibroblast; end., endothelial cells of capillaries; l., lymphocytes; lkc., granular leukocytes; p.c., plasma cell; r.b.c., red blood cells; ret., reticular fibers; s., septal cells. Mallory-azan stain. Leitz 2 mm. apochromatic objective and × 12 ocular. Camera lucida at stage level.

a.ph.
p.c.
f b.
al.
ret.
al.
end.
al.
lkc.
l.
s.
lkc.
end.
l.
al.
al.
r.b.c.
r.b.c.

PLATE 2

Section of a collapsed rabbit lung removed 5 days after phrenicotomy and first pneumothorax: al., collapsed alveolus; a.ph., alveolar phagocyte; end., endothelial cells of capillaries; fb., fibroblast; l., lymphocytes; lkc., granular leukocytes; pl., pleura; r.b.c., red blood cells; ret., reticular fibers; s, septal cells; ven., venule; pl., pleura. Foot's modification of Bielschowsky's reticulum stain and Mallory-azan stain. Leitz 2 mm. apochromatic objective and × 10 ocular. Camera lucida at stage level.


end.
end.
end.
ret.
al.
a.ph.
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fb.
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r.b.c.
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pl.
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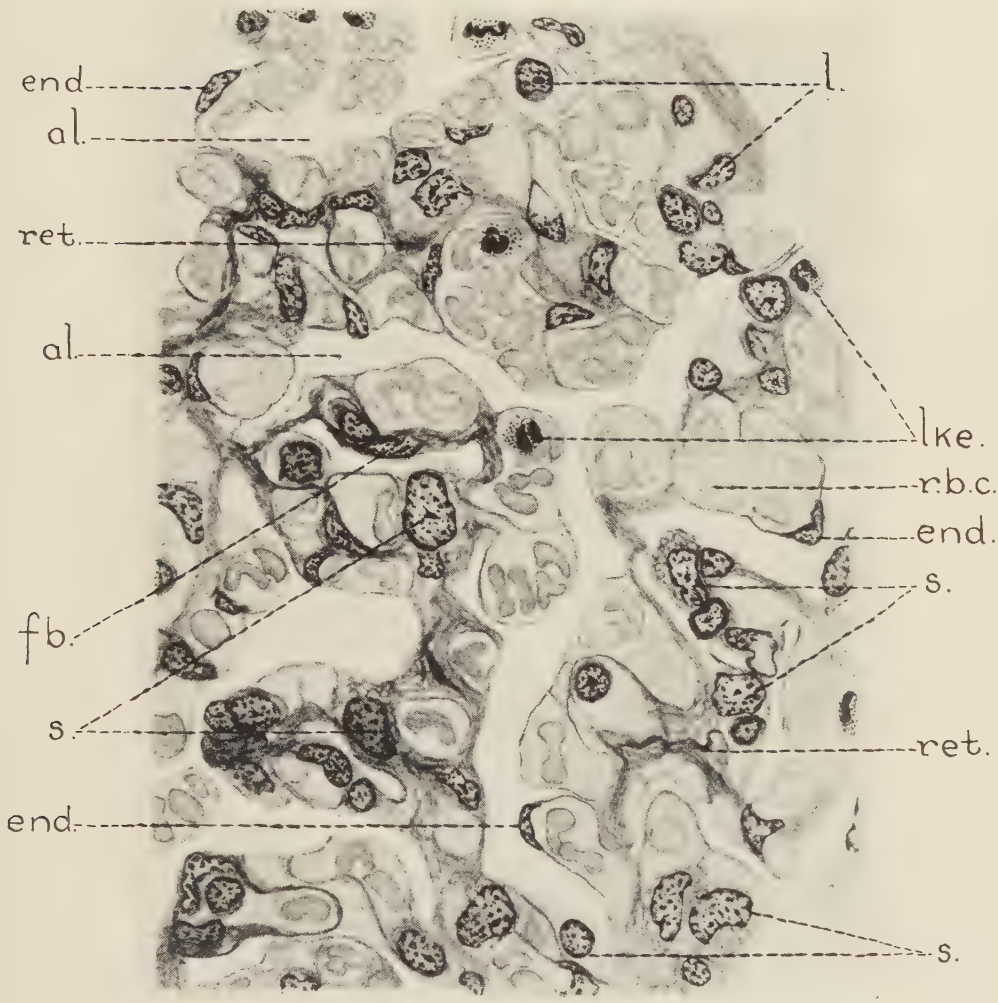
ret.
l.
al.
al.

PLATE 3

Section of collapsed rabbit lung removed 10 days after phrenicotomy and first pneumothorax: al., collapsed alveolus; end., endothelial cells; fb., fibroblast; l., lymphocytes; lke., granular leukocytes; r.b.c., red blood cells; ret., reticulum; s., septal cells. Hematoxylin-anilin blue-orange G stains. Leitz 2 mm. apochromatic objective and × 15 ocular. Camera lucida at stage level.


end
al.
ret.
al.
fb.
s.
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l.
lke.
r.b.c.
end.
s.
ret.
s.

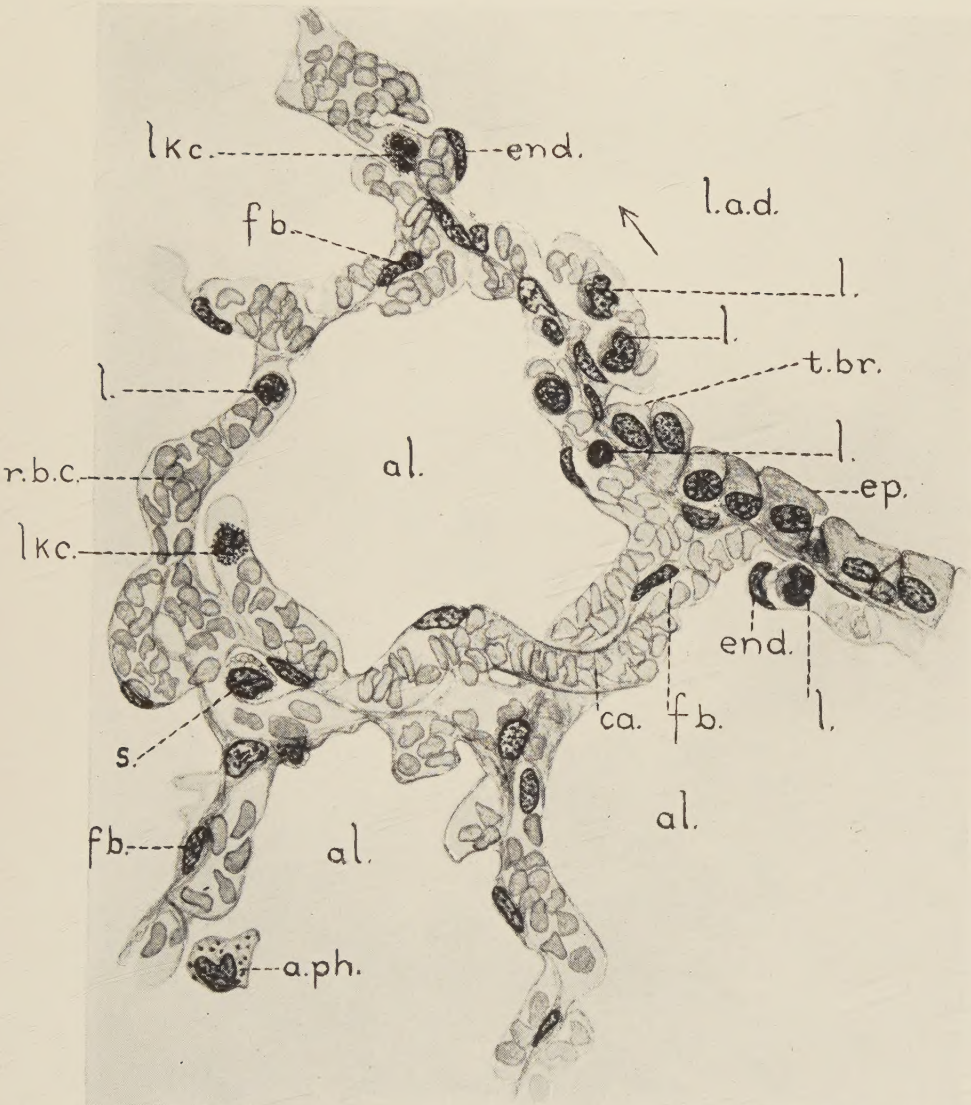
PLATE 4

Section of collapsed rabbit lung removed 15 days after phrenicotomy and first pneumothrax: al., collapsed alveolus; al.d., alveolar duct; a.ph., alveolar phagocytes; art., arteriole; end., endothelial cells of capillaries; ep., epithelium of bronchiole; fb., fibroblasts; l., lymphocytes; lkc., granular leukocytes; r.b.c., red blood cells; ret., reticulum; s., septal cells; s.m., smooth muscle cells; t.br., termination of bronchiole. Mallory-azan stain. Leitz 2 mm. apochromatic objective and × 10 ocular. Camera lucida at stage level.

r.b.c
a l.
ep
art.
lke.
ald
s.
sm
aph.
ret
end.
t br
al.
ret.
s.
lke.
s.
l.
end
l.
s.

PLATE 5

Section of expanded rabbit lung. The capillaries were filled with blood by clamping the pulmonary veins before the animal was killed: al., alveolus; ca., capillary crossing from one side of the septum to the other; a.ph., alveolar phagocyte; end., endothelial cells of capillaries; ep., epithelium of bronchiole; fb., fibroblasts; l., lymphocytes; l.a.d., lumen of alveolar duct; lkc., granular leukocytes; r.b.c., red blood cells; s., septal cells; t.br., termination of bronchiole. Hematoxylin-eosin-azure II stain. Leitz 2 mm. apochromatic objective and × 10 ocular. Camera lucida at stage level.


lkc.
end.
l.a.d.
fb.
l.
l.
t.br.
l.
al.
l.
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lkc.
end.
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al.
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